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Pesticide Use and Incident Hypothyroidism in Pesticide Applicators in the Agricultural Health Study

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Background: Though evidence suggests that some pesticides may have thyroid-disrupting properties, prospective studies of associations between specific pesticides and incident thyroid disease are limited.

Objective: We evaluated associations between use of specific pesticides and incident hypothyroidism in private pesticide applicators in the Agricultural Health Study (AHS).

Methods: Self-reported incident hypothyroidism ($n = 829$ cases) was studied in relation to ever-use and intensity-weighted cumulative days of pesticide use at study enrollment. We estimated adjusted hazard ratios (HRs) and 95% confidence intervals (CI) using Cox proportional hazards models applied to 35,150 male and female applicators followed over 20 y. All models were stratified by state and education to meet proportional hazards assumptions ($p \leq 0.10$ for age x covariate interactions). Models of pesticides that did not meet proportional hazards assumptions were stratified by median attained age (62 y).

Results: Hypothyroidism risk was significantly increased with ever- vs. never-use of four organochlorine insecticides (aldrin, heptachlor, and lindane among participants with attained age >62 y; chlordane in all participants), four organophosphate insecticides (coumaphos in those >62 y; diazinon, dichlorvos, and malathion in all participants) and three herbicides (dicamba, glyphosate, and 2,4-D in all participants). HRs ranged from 1.21; 95% CI: 1.04, 1.41 (chlordane) to 1.54; 95% CI: 1.23, 19.4 (lindane in those >62 y). Hypothyroidism risk was greatest among those with higher intensity-weighted lifetime days of using chlordane, lindane, coumaphos (over age 62), diazinon, permethrin, and 2,4-D.

Conclusions: Our findings support associations between exposure to several pesticides and increased hypothyroidism risk. These findings are generally consistent with prior analyses of prevalent hypothyroidism in the AHS. https://doi.org/10.1289/EHP3194

Introduction

Thyroid diseases are common in the United States (U.S.); and with prevalence estimates of 5–9% (overt and subclinical disease combined), hypothyroidism is the most common thyroid disease (Garber et al. 2012). Some of the known nonmodifiable risk factors for thyroid diseases in general include increasing age, female sex, a history of autoimmune diseases, and a family history of thyroid disease; modifiable risk factors include suboptimal iodine intake and some medications (Sawin 2005). Optimal thyroid hormone (TH) levels are important to many physiological processes, and thyroid dysfunction is associated with multiple adverse health outcomes, including cardiovascular diseases, neuropsychiatric disorders, and poor bone and reproductive health (Cooper and Biondi 2012; Klein and Ojamaa 2001; Whybrow and Bauer 2005a, 2005b). Identification of modifiable risk factors thus may have important public health implications.

Both toxicological and human studies suggest associations between several pesticides and thyroid-related alterations (Campos and Freire 2016). Of more relevance, human studies have reported associations between specific pesticides [for example, organochlorine insecticides dichlorodiphenyltrichloroethane (DDT) and aldrin, and the fungicide maneb] and subtle changes in circulating levels of THs and thyroid stimulating hormone (TSH) (Blanco-Muñoz et al. 2016; Campos and Freire 2016; Freire et al. 2013; Steenland et al. 1997), including a recent investigation among a subset of Agricultural Health Study (AHS) participants (Lerro et al. 2017). However, findings from these studies have been somewhat inconsistent, and they were conducted mainly in euthyroid populations; as such, they do not provide direct information on clinical thyroid conditions.

Only a handful of epidemiologic studies have examined associations between pesticides and clinical thyroid diseases, including two evaluations of prevalent thyroid disease within the AHS (Goldner et al. 2010, 2013). The findings suggest higher prevalence of hypothyroidism in male private pesticide applicators exposed to the chlorophenoxy herbicides and some organochlorine and organophosphate insecticides, and higher prevalence of hypothyroidism in their female spouses who reported ever-use of the fungicides maneb/mancozeb and benomyl. Both prevalent and incident hypothyroidism cases were included but not distinguished in the prior analyses, so limited inference could be made regarding temporality of exposures and outcomes. To address this limitation, we examined associations between pesticide use and incident hypothyroidism in private pesticide applicators in the AHS, including newly reported cases of hypothyroidism from an extended follow-up as well as incident cases from prior surveys.

Methods

Study Population

The AHS is a prospective cohort study of licensed pesticide applicators (mainly farmers) and their spouses from North Carolina and Iowa (Alavanja et al. 1996). Between 1993–1997 (Phase 1), individuals renewing or applying for licenses to use restricted pesticides were invited to enroll by completing a

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questionnaire at a pesticide licensing location. The enrollment questionnaire asked about sociodemographic characteristics, pesticide use, work practices, and medical history. The questionnaire was completed and returned by 52,394 private pesticide applicators (84% of those invited). The enrolled farmers were provided an additional take-home questionnaire (completed by 22,916 participants, representing 44% of those completing the enrollment questionnaire) that obtained additional details on pesticide use and practices. Follow-up interviews were conducted in 1999–2003 (Phase 2), 2005–2010 (Phase 3), and 2013–2016 (Phase 4) to update sociodemographic and medical information. All questionnaires can be accessed from the study website (https://aghealth.nih.gov/collaboration/questionnaires.html). We restricted our analysis to farmers who completed at least one follow-up survey ($N = 38{,}698$), but we evaluated the impact of loss-to-follow-up in a sensitivity analysis. This study was approved by all applicable Institutional Review Boards.

Pesticide use. On the enrollment questionnaire, we asked about ever-use of 50 individual pesticides, and duration (number of years) and frequency (average days per year) of use for 22 of these pesticides. On the take-home questionnaire, we asked about duration and frequency of use for the remaining 28 pesticides. These questionnaires also asked for detailed information on pesticide application methods, whether the applicator mixed pesticides and repaired the equipment used to mix or apply pesticides, and use of personal protective equipment (PPE), which were incorporated into an exposure intensity weighting algorithm. Details on the algorithm construction are described elsewhere (Coble et al. 2011; Dosemeci et al. 2002). Very briefly, exposure intensity scores were assigned depending on general exposure scenarios. Higher scores were assigned for inadequate PPE use, involvement in the repair of pesticide application equipment, or pesticide application methods resulting in higher exposure, such as air blast or hand spray; lower scores were assigned for adequate PPE use, better personal hygiene habits (changing or washing right away after pesticide use, or using disposable clothing), and pesticide application methods resulting in lower exposure. An algorithm was applied to obtain overall exposure intensity score for each participant. The validity of the algorithm has been evaluated using biomonitoring data from a substudy of pesticide applicators (Coble et al. 2005; Thomas et al. 2010). To calculate lifetime days of use for each pesticide, we multiplied "years of use" by "average days per year of use"; these were then multiplied by an intensity-weighting factor to obtain intensity-weighted lifetime days of use.

We used two exposure metrics: *a*) ever- vs. never-use; and *b*) intensity-weighted lifetime days of use with multiple categories depending on sample size availability. We restricted our analysis to pesticides with at least 10 hypothyroidism cases in each exposure category. We used four categories of intensity-weighted lifetime days (never-use, and three categories based on tertiles of intensity-weighted days among users); however, when we did not have sufficient sample size, we used three categories (never-use, ≤median, and >median days among users).

Thyroid disease. Participants were asked about doctor-diagnosed thyroid diseases in all four phases of the AHS (see Table S1). Briefly, on the Phase 1 take-home questionnaire, participants were asked if they had goiter, thyrotoxicosis/Grave's disease, or other thyroid disease, and the age range during which disease was diagnosed. Phase 1 thyroid disease information was not available for those not completing the take-home questionnaire. In Phase 2, more detailed information was collected; participants were asked if they were ever told by a doctor that they had *a*) thyroid disease; *b*) an underactive thyroid (hypothyroidism) and if that condition was due to thyroiditis, Hashimoto's disease or autoimmune disease, or other causes; *c*) an overactive thyroid (hyperthyroidism) and if that condition was due to Grave's disease, thyrotoxicosis, or other causes; *d*) an enlarged thyroid, thyroid nodules or Goiter; or *e*) some other thyroid problems. They were also asked about the age they were first diagnosed with that condition, and if they ever received medication or treatment for each of those conditions. In Phases 3 and 4, they were asked questions similar to those in Phase 2.

Figure S1 provides a detailed description of overall sample selection. Of 38,698 who completed at least one follow-up survey, we excluded 217 who did not respond to thyroid disease questions in any of the surveys, 374 who reported having thyroid disease on the Phase 1 take-home questionnaire (prevalent cases, diagnosed before Phase 2), 81 thyroid cancer cases (identified from cancer registry linkage), 210 cases who provided "inconsistent" responses (i.e., reported having thyroid disease in a preceding survey but did not report having it in a subsequent survey), and 33 cases who reported having other conditions (for instance, congenital thyroid disease, benign cysts, and nonthyroid conditions such as pituitary tumors, etc.), leaving 37,783 participants. Of these, 2,041 reported any thyroid disease at follow-up: 1,546 were incident cases (age at diagnosis >age at enrollment); 388 reported age at diagnosis ≤age at enrollment (prevalent cases), and 107 lacked information on whether thyroid disease was diagnosed before or after enrollment. The 495 cases in these last two groups were excluded.

Hypothyroidism

Thyroid diseases, specifically subclinical conditions, can revert to euthyroid status over time (Somwaru et al. 2012; Zhyzhneuskaya et al. 2016). Further, hypothyroidism sometimes can develop after hyperthyroidism, as a natural course of disease (for instance, persistent or transient hypothyroidism after subacute thyroiditis), or often after treatment for hyperthyroidism, including thyroidectomy and radioiodine treatment (Chaker et al. 2017). Hypothyroidism has also been reported to convert to hyperthyroidism, likely due to presence of different autoantibodies, including both thyroid stimulating and blocking antibodies (Furqan et al. 2014). Possibly due to the variable course of disease or to misreporting, some participants reported multiple thyroid disease types within a survey and different types across surveys. Decision rules were applied to define "hypothyroidism" and "hyperthyroidism" for these individuals (see Table S2).

Briefly, when participants reported having hyperthyroidism and hypothyroidism in the same survey (or in separate surveys) with diagnosis age for hyperthyroidism preceding that of hypothyroidism, participants were considered to have developed hyperthyroidism first, with the assumption that their hypothyroidism resulted from treatment or natural course of hyperthyroidism. On the other hand, if participants reported that hypothyroidism preceded hyperthyroidism, they were candidates for exclusion, with some exceptions (Table S2).

Of 1,546 incident thyroid disease cases, 870 were hypothyroid, 279 were hyperthyroid, 69 were categorized as other/goiter/inconsistent, and 328 had no information on thyroid disease type ("unspecified"). Other/goiter/inconsistent and unknown thyroid diseases were excluded from analyses. The final sample included 35,150 participants [34,050 without thyroid disease, 829 with hypothyroidism, and 271 with hyperthyroidism (note: those who developed hyperthyroidism were censored at diagnosis)] who completed the enrollment questionnaire and at least one follow-up survey, after excluding individuals without complete information on covariates (i.e., age, gender, state, education, and smoking). Thus, ever-never use analyses and intensity-weighted analyses for the 22 pesticides for which frequency and duration of use were asked in

the enrollment questionnaire were completed in the 35,150-person sample. However, for intensity-weighted analyses for the 28 pesticides for which frequency and duration of use were asked only in the take-home questionnaire, the final sample was 17,832 (506 with hypothyroidism).

Age at diagnosis of thyroid disease. Some participants had inconsistencies in reported age at diagnosis. We used age at diagnosis provided at the earliest survey reporting a diagnosis and age, as people may recall less well with time. If participants did not report age at diagnosis when first reporting a diagnosis, then we used age at diagnosis reported in a subsequent survey. For participants who did not provide an age at diagnosis, we estimated age at diagnosis as the midpoint between the last disease-free phase and when the disease was first reported. Age at diagnosis was estimated for 45 (4%) of the hypothyroidism cases.

Thyroid disease validation. We carried out a small validation effort to assess the quality of self-reported data. Participants who had reported incident thyroid disease during the third and fourth follow-up survey were recontacted to confirm their diagnosis, provide details of medication use and treatments, and to provide permission for retrieval of medical records. To date, we have obtained completed disease confirmation questionnaires from 1,146 participants (applicators and spouses) who reported any thyroid disease. Of the 819 with self-reported hypothyroidism who completed a questionnaire, 82% affirmed their hypothyroidism diagnosis, whereas only 51% of those with hyperthyroidism (or both) ($n=216$) who completed the questionnaire affirmed the diagnosis. Medical records were obtained for 162 self-reported hypothyroidism and 37 hyperthyroidism (or both) cases. Physicians/medical staff confirmed the diagnosis of hypothyroidism 90% of the time. However, only 32% of self-reported hyperthyroidism cases were confirmed by medical records, possibly because we did not reach the diagnosing physician or because some of these were currently being treated for hypothyroidism and office staff may not have searched the entire medical record (our own nonsystematic review of several complete records that were sent to us suggest that this circumstance may be the case).

Statistical analysis. We used logistic regression to assess associations between covariates and hypothyroidism and report odds ratios and 95% confidence intervals (CIs). We used Cox proportional hazards models to estimate hazard ratios (HRs) and their 95% CIs for associations of pesticides with hypothyroidism. We used attained age as the time scale with left truncation at enrollment and adjusted for sex, education, state of residence, and smoking. We selected these covariates for confounding adjustment *a priori* based on their potential causal relations with pesticide use and thyroid disease as identified from prior literature and current observations using causal diagrams (Greenland et al. 1999). Time at risk was accrued until hypothyroidism or hyperthyroidism diagnosis, death, and loss or end of follow-up. We tested for proportional hazards assumptions using an interaction term between attained age and covariates. The covariates state of residence and education failed to meet the proportional hazards assumptions (p for proportionality ≤ 0.10). Therefore, we used state- and education-stratified Cox models to allow baseline hazards to vary within the strata of these covariates. For any pesticides that failed to meet proportional hazards assumptions, we allowed hazards to vary by the median attained age (i.e., 62 y). For the intensity-weighted analysis, we also estimated $p\text{-}_{\text{trend}}$ using the median value for each exposure category as an ordinal variable in regression models.

We performed several sensitivity analyses for hypothyroidism—adjusting for correlated pesticides (Phi coefficient: ≥ 0.40); restricting cases of hypothyroidism ($n=752$) to those who reported receiving medication or treatment in at least one survey; and excluding 775 female applicators ($n=34{,}375$ for overall sample).

We also conducted additional sensitivity analyses in light of the validation data: restricting cases to those who consistently reported having a hypothyroidism diagnosis at least twice across surveys, or who reaffirmed the diagnosis or indicated they had treatments appropriate to their conditions in a disease confirmation questionnaire, or were confirmed by medical records (overall $n=34{,}464$ with 346 hypothyroidism and 68 hyperthyroidism cases; $n=686$ excluded, which includes 483 hypothyroidism and 203 hyperthyroidism cases).

We investigated the impact of loss to follow-up on our findings as 13,696 farmers did not participate in any of the three follow-up surveys, and 14,553 who participated in Phase 2 and/or Phase 3 did not participate in Phase 4 (with a total of 28,249 nonrespondents in Phase 4). For this, we repeated the analysis of ever-use of pesticides and hypothyroidism risk, employing inverse probability of censoring weights (Cole and Hernán 2008; Robins et al. 2000). Briefly, we used weighted Cox models to estimate HRs and 95% CIs, adjusting for covariates and using stabilized weights. For stabilized weight estimation, first we transformed our data from a single record per person into person–year data [i.e., multiple records per person with number of records depending on time (i.e., years) at risk]. Then, we used logistic regression analyses on the transformed data to estimate two different predicted conditional probabilities of overall participation in Phase 4. The first, used to calculate the denominator of the stabilized weights, is conditional on exposure, year, and baseline covariates (age, gender, education, smoking, owned farm, cumulative exposure to any pesticides, state of residence, and pair-wise interaction between state and covariates). The second, used to calculate the numerator of the stabilized weights, is conditional only on year. Finally, we estimated stabilized weights as the ratio of cumulative conditional probabilities. We also evaluated two additional covariates, cumulative exposure to any pesticides and owned farm, as predictors of loss to follow-up. Because these variables were missing for some participants (a total of 1,723 of the 35,150-person sample had missing values for either of the covariates), we used multiple imputation to impute values for these two covariates. Briefly, we used the fully conditional specification method to impute missing covariates (Lee and Carlin 2010), created five imputed datasets, performed regression analysis in each dataset, and obtained the pooled parameter estimates. We used AHS data releases AHSREL20150600, P1REL201209_00, P2REL20120900, P3REL20120900, and P4PreRel06172015. Analyses were performed using SAS version 9.4 (SAS Inc.).

Results

Some baseline characteristics of participants varied by thyroid disease status (Table 1). In comparison with those individuals who did not develop hypothyroidism, those who developed hypothyroidism were older, more likely to be female and from Iowa, and they were less likely to have consumed alcohol in the year before enrollment and tended to have higher education. Those respondents who reported "currently smoking" at baseline were less likely to develop hypothyroidism.

After adjusting for sex, state, education, and smoking, ever-use of four organochlorine insecticides (aldrin, heptachlor, and lindane among participants with attained age >62 y; chlordane in all participants), four organophosphate insecticides (coumaphos in those aged >62 y; diazinon, dichlorvos, and malathion in all participants), and three herbicides [dicamba, glyphosate, and 2,4-dichlorophenoxyacetic acid (2,4-D) in all participants] was significantly associated with elevated risk of hypothyroidism (Table 2). The HRs ranged from 1.21; 95% CI: 1.04, 1.41 (for chlordane) to

Table 1. Baseline characteristics of the participants ($N = 34,879$).

Characteristics	Non-cases ($n = 34,050$)	Hypothyroidism ($n = 829$)	
	n (%)	n (%)	OR (95% CI)[a]
Age			
≤45 years	17,009 (50)	307 (37)	Ref
46–55 years	8,086 (23.7)	232 (28)	1.58 (1.32, 1.89)
56–65 years	6,226 (18.3)	208 (25.1)	1.97 (1.63, 2.39)
>65 years	2,729 (8)	82 (9.9)	1.78 (1.37, 2.33)
Gender			
Women	706 (2.1)	48 (5.8)	Ref
Men	33,344 (97.9)	781 (94.2)	0.32 (0.24, 0.44)
State of residence			
Iowa	22,223 (65.3)	577 (69.6)	Ref
North Carolina	11,827 (34.7)	252 (30.4)	0.70 (0.59, 0.83)
Education			
≤High school graduate	19,165 (56.3)	400 (48.3)	Ref
1–3 years beyond high school	8,585 (25.2)	231 (27.9)	1.39 (1.17, 1.66)
College graduate or more	6,300 (18.5)	198 (23.9)	1.68 (1.40, 2.01)
Smoking status			
Never smoker	18,810 (55.2)	450 (54.3)	Ref
Former smoker	10,315 (30.3)	295 (35.6)	1.18 (1.01, 1.38)
Current smoker	4,925 (14.5)	84 (10.1)	0.84 (0.66, 1.08)
Alcohol consumption (past year)[b]			
No	10,933 (33.3)	311 (38.6)	Ref
Yes	21,907 (66.7)	494 (61.4)	0.79 (0.67, 0.92)

[a]Estimated using logistic regression ($n = 271$ hyperthyroidism cases not included in the model).
[b]$n = 1,210$ missing for noncases, and $n = 24$ missing for hypothyroidism.
Note: OR, Odds Ratio; CI, Confidence Intervals.

1.54; 95% CI: 1.23, 1.94 (for lindane among those ages >62 y). After adjusting for correlated pesticides (Phi coefficient ≥0.40), associations remained significant for chlordane (adjusted for DDT), heptachlor among those ages >62 y (adjusted for aldrin and dieldrin), diazinon (adjusted for carbaryl), and dicamba (adjusted for imazethapyr), but not aldrin among those ages >62 y (adjusted for DDT, dieldrin, and heptachlor, HR = 1.15; 95% CI: 0.87, 1.53) (Table S3). Phi coefficients were <0.40 for the other significantly associated pesticides. Carbamate and pyrethroid insecticides, fungicides, and fumigants were not significantly associated with hypothyroidism.

Table 3 shows the results for analyses using intensity-weighted lifetime days for insecticides and fumigants. Significant linear dose-response trends (p-trend ≤0.05) were seen for chlordane, lindane, and diazinon in all participants, and for coumaphos among those over age 62 y. There were suggestive results (p-trend ≤0.20) for aldrin, dichlorvos, fonofos, and permethrin (used on both animals and crops), although HRs across increasing exposure categories did not always show monotonic increases for these pesticides. Table 4 shows the intensity-weighted results for fungicides and herbicides. Increasing hypothyroidism risk with increasing level of exposure was seen for 2,4-D in the overall sample (p-trend = 0.01). There was also a significant (p-trend = 0.05) for 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) among older applicators, although HRs increased only slightly between the second and third exposure category. Although there was not a significant dose–response for glyphosate, HRs were significantly elevated in the second (HR = 1.27, 95% CI: 1.03, 1.69) and third (HR = 1.38; 95% CI: 1.12, 1.69) level of intensity-weighted days. There was also suggestive evidence for dose–response for increasing hypothyroidism risk for dicamba in the overall sample and for atrazine among those >62 y (p-trend ≤0.20), and decreasing hypothyroidism risk with 2,4,5-T among those ≤62 y and metolachlor and chlorothalonil in the overall sample (p-trend ≤0.20). When adjusted for correlated pesticides wherever applicable, $p\text{-}_{\text{trend}}$ for chlordane (adjusted for DDT), diazinon (adjusted for carbaryl), and dicamba (adjusted for imazethapyr) in the overall sample and 2,4,5-T (adjusted for 2,4,5-TP) for both age groups (≤62 y and >62 y) remained similar with very similar point estimates (Table S4); $p\text{-}_{\text{trend}}$ for chlorothalonil (adjusted for aldicarb and benomyl) was 0.39. No analysis for aldrin was done as the proportional hazards assumption was not met (when adjusted for correlated pesticides) and sample size was not adequate for stratification by age. The other pesticides that we noted to have suggestive associations in the main analysis were not correlated with other pesticides.

The results for hypothyroidism were similar in sensitivity analyses—when hypothyroidism was defined as those who received medication/treatment (Table S5); and when female applicators were excluded (Table S6). In the analysis restricting cases to those reporting hypothyroidism at least two times across surveys, and/or confirmed by a validation questionnaire or medical records (overall $n = 34,464$; 346 hypothyroidism and 68 hyperthyroidism (censored at diagnosis) cases included in the analysis), the organochlorines chlordane (in the overall sample) and lindane (among those ages >62 y) were significantly associated with hypothyroidism risk (Table S7). However, for remaining organochlorines evaluated, in contrast to the primary analysis, we no longer observed interaction with age and HRs in the overall sample were generally close to one. The organophosphate coumaphos was no longer significantly associated with hypothyroidism (HR = 0.88; 95% CI: 0.60, 1.30, in all participants), but diazinon, dichlorvos, and malathion remained positively associated, with stronger HRs in comparison with those in the main analysis. For diazinon, in contrast with the main analysis, HRs were allowed to vary by the median age of 62 y and were significantly elevated in both strata. In the analysis that used inverse probability of censoring weights, the point estimates were very similar to the main analysis for all the pesticides evaluated; toxaphene, aluminum phosphide, and atrazine were not stratified by the median age in this analysis in contrast with the main analysis although the point estimates were consistent (Table S8).

Discussion

In this large prospective cohort of farmers that were occupationally exposed to pesticides, we found that ever-use of four organochlorine insecticides (aldrin, chlordane, heptachlor, and

Table 2. Ever-use of pesticides and hypothyroidism risk.

Pesticide	Exposed cases	HR (95% CI)[a]	*P*-value
Organochlorine insecticide			
Aldrin ≤62 years[b]	57	0.87 (0.65, 1.17)	0.36
>62 years[b]	158	1.28 (1.02, 1.60)	0.03
Chlordane	280	1.21 (1.04, 1.41)	0.02
DDT ≤62 years[b]	63	0.84 (0.64, 1.12)	0.24
>62 years[b]	207	1.18 (0.95, 1.47)	0.13
Dieldrin ≤62 years[b]	19	0.95 (0.59, 1.51)	0.81
>62 years[b]	69	1.22 (0.93, 1.60)	0.14
Heptachlor ≤62 years[b]	44	0.80 (0.58, 1.11)	0.19
>62 years[b]	140	1.35 (1.07, 1.70)	0.01
Lindane ≤62 years[b]	91	1.01 (0.80, 1.28)	0.91
>62 years[b]	118	1.54 (1.23, 1.94)	<0.01
Toxaphene ≤62 years[b]	44	0.78 (0.56, 1.07)	0.12
>62 years[b]	85	1.14 (0.89, 1.46)	0.31
Carbamate insecticide			
Aldicarb	61	0.76 (0.58, 1.01)	0.06
Carbaryl	502	1.13 (0.97, 1.32)	0.12
Carbofuran	258	1.12 (0.97, 1.31)	0.13
Organophosphate insecticide			
Chlorpyrifos	351	1.02 (0.89, 1.18)	0.75
Coumaphos ≤62 years[b]	49	0.88 (0.63, 1.24)	0.47
>62 years[b]	36	1.44 (1.06, 1.95)	0.02
Diazinon	312	1.27 (1.10, 1.48)	<0.01
Dichlorvos	131	1.42 (1.17, 1.72)	<0.01
Fonofos	205	1.15 (0.97, 1.36)	0.11
Malathion	629	1.23 (1.04, 1.46)	0.02
Parathion	142	1.18 (0.97, 1.42)	0.10
Phorate	289	1.02 (0.88, 1.19)	0.77
Terbufos	336	1.14 (0.98, 1.32)	0.09
Pyrethroid insecticide			
Permethrin (for animals)	126	1.20 (0.99, 1.46)	0.07
Permethrin (for crops)	116	1.19 (0.98, 1.46)	0.08
Fumigant			
Carbon tetrachloride/Carbon disulphide 80/20 mix	58	1.00 (0.76, 1.32)	0.98
Aluminum Phosphide ≤62 years[b]	23	0.96 (0.63, 1.46)	0.83
>62 years[b]	18	1.26 (0.79, 2.04)	0.32
Ethylene Dibromide	23	0.79 (0.52, 1.20)	0.26
Methyl Bromide	113	0.96 (0.76, 1.21)	0.71
Fungicide			
Benomyl	76	0.93 (0.72, 1.21)	0.59
Captan	87	0.91 (0.73, 1.14)	0.42
Chlorothalonil	53	0.92 (0.69, 1.24)	0.59
Maneb/Mancozeb	75	0.95 (0.73, 1.23)	0.69
Metalaxyl	158	0.91 (0.75, 1.11)	0.35
Herbicide			
Alachlor	448	1.05 (0.91, 1.22)	0.51
Butylate	283	1.09 (0.94, 1.27)	0.26
Chlorimuron Ethyl	263	0.93 (0.80, 1.08)	0.35
Dicamba	455	1.27 (1.08, 1.50)	<0.01
EPTC	171	1.08 (0.91, 1.29)	0.37
Glyphosate	663	1.28 (1.07, 1.52)	0.01
Imazethapyr	343	1.01 (0.86, 1.18)	0.96
Metolachlor	364	0.98 (0.85, 1.13)	0.80
Paraquat	170	0.91 (0.76, 1.10)	0.33
Pendimethalin	312	0.90 (0.78, 1.04)	0.15
Petroleum Oil ≤62 years[b]	207	0.92 (0.76, 1.11)	0.40
>62 years[b]	184	1.21 (0.97, 1.51)	0.09
Trifluralin	449	1.11 (0.95, 1.30)	0.18
2 4-D	671	1.30 (1.07, 1.58)	0.01
2,4,5-T	219	1.06 (0.90, 1.24)	0.52
2,4,5-T P	94	1.11 (0.89, 1.39)	0.34
Atrazine ≤62 years[b]	316	0.89 (0.72, 1.11)	0.30
>62 years[b]	287	1.18 (0.91, 1.51)	0.21
Cyanazine	362	1.09 (0.93, 1.27)	0.29
Metribuzin	379	1.02 (0.88, 1.19)	0.80

[a]Adjusted for sex, education, state, and smoking.
[b]Hazard ratio allowed to vary by the median age (i.e., 62 years) for pesticides that did not meet proportional hazards assumptions ($p \le 0.10$).
Note: "Never exposed" to a pesticide is a comparison group, and those who developed hyperthyroidism were censored at age of diagnosis. 2,4-D, 2,4-Dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-Trichlorophenoxyacetic acid; 2,4,5-T,P, 2-(2,4,5-trichlorophenoxy) propionic acid; CI, Confidence Intervals; DDT, Dichlorodiphenyltrichloroethane; EPTC, S-Ethyl dipropylthiocarbamate; HR, Hazard Ratio.

lindane), four organophosphate insecticides (coumaphos, diazinon, dichlorvos, and malathion), and three herbicides (dicamba, glyphosate, and 2,4-D) was associated with increased risk of hypothyroidism. These findings are consistent with the previous investigation in the AHS male farmers that examined *any* hypothyroidism (prevalent and incident cases combined ($n = 461$) identified through Phase 3) (Goldner et al. 2013). Our current analysis expanded on this earlier report by focusing only on incident disease and including five more years of follow-up data (an additional 486 incident hypothyroidism cases were reported in Phase 4) as well as by including female farmers.

We observed elevated hypothyroidism risk in those reporting ever-use of the organochlorines aldrin, heptachlor, and lindane among those aged >62 y and the organochlorine chlordane in the overall sample. In intensity-weighted analyses, we observed greater risks in those exposed to the top two higher exposure categories of chlordane and lindane. Organochlorine insecticides were extensively used in the United States in the 1950s, but most of these insecticides have been banned for use since the 1980s (Jones and de Voogt 1999). Specifically, aldrin and chlordane were banned in 1974 and 1983, respectively (ATSDR 1994; 2002), and although lindane was not banned until 2006, it was already being voluntarily phased out in earlier years for most uses (U.S. EPA 2006). Nevertheless, these pesticides can still be detected in the general U.S. population due to their persistent and bioaccumulative nature (CDC 2009; Jones and de Voogt 1999). Therefore, observed age-specific associations are likely explained by the possibility that older participants have had higher and more sustained exposures to these insecticides than younger participants did. Further, increasing age is an important risk factor for hypothyroidism (Sawin 2005). Lack of age-specific associations in analyses restricted to cases reporting hypothyroidism at least two times and to confirmed cases could be explained by the fact that excluded cases were older than those included. Ever-use of most organochlorines, including chlordane, DDT, heptachlor, lindane, and toxaphene, were positively associated with hypothyroidism in the previous study of the AHS farmers; that study, however, did not report age-specific associations (Goldner et al. 2013). Ever-use of any organochlorine was also associated with higher prevalence of hypothyroidism in the female spouses of AHS farmers, but associations for specific organochlorines, although elevated, were not statistically significant (Goldner et al. 2010). A recent investigation in the Biomarkers of Exposure and Effect in Agriculture (BEEA) study, a molecular epidemiologic substudy nested within the AHS, examined pesticide use in relation to thyroid function biomarkers in 679 male farmers without thyroid disease and not using thyroid medications; prior use of the organochlorine aldrin was associated with increased prevalence of subclinical hypothyroidism (defined as TSH >4.5 mIU/L) after adjustment for correlated pesticides (Lerro et al. 2017). In the present study, ever-use of aldrin was also associated with increased risk of hypothyroidism among participants aged >62 y, although the HR estimate was closer to the null and no longer statistically significant when adjusted for correlated organochlorines. Several other smaller cross-sectional studies have also reported associations between serum levels of organochlorine insecticides, including chlordane, DDT or its metabolite dichlorodiphenyldichloroethylene (DDE), dieldrin, and heptachlor, and serum THs or TSH concentrations; however, the directions of associations are not consistent across the studies and, in some instances, are shown to differ by sex (Blanco-Muñoz et al. 2016; Freire et al. 2013; Piccoli et al. 2016).

Our findings also suggest associations between ever-use of four organophosphates and higher hypothyroidism risk, although increases in intensity and duration of the exposures were

Table 3. Intensity-weighted lifetime days of use of insecticides and fumigants and hypothyroidism risk.

Pesticide	Intensity-weighted days[a]	Cases	HR (95% CI)[b]	*p*-Value	*p*-Trend[c]
Organochlorine insecticide					
Aldrind	Unexposed	361	Ref		0.06
	>0 – ≤315	47	1.34 (0.97, 1.84)	0.07	
	>315 – ≤980	41	1.24 (0.88, 1.73)	0.21	
	>980	44	1.38 (0.99, 1.91)	0.06	
Chlordaned	Unexposed	347	Ref		0.01
	>0 – ≤238	42	1.30 (0.94, 1.80)	0.11	
	>238 – ≤720	53	1.53 (1.14, 2.05)	0.01	
	>720	48	1.49 (1.09, 2.04)	0.01	
DDT[d]	Unexposed	344	Ref		0.47
	>0 – ≤335	48	1.06 (0.77, 1.45)	0.73	
	>335 – ≤1599	51	1.10 (0.80, 1.49)	0.56	
	>1599	48	1.13 (0.82, 1.55)	0.45	
Heptachlor[d]	Unexposed	409	Ref		0.74
	>0 – ≤289	26	0.99 (0.66, 1.48)	0.95	
	>289 – ≤882	29	1.10 (0.75, 1.62)	0.62	
	>882	26	1.05 (0.70, 1.58)	0.80	
Toxaphene[d]	Unexposed	425	Ref		0.69
	>0 – ≤315	24	1.08 (0.71, 1.63)	0.73	
	>315 – ≤1176	27	1.41 (0.95, 2.08)	0.09	
	>1176	15	0.83 (0.49, 1.40)	0.48	
Lindaned	Unexposed	391	Ref		0.01
	>0 – ≤315	29	1.10 (0.75, 1.61)	0.62	
	>315 – ≤1176	36	1.45 (1.02, 2.04)	0.04	
	>1176	36	1.52 (1.08, 2.14)	0.02	
Carbamate insecticide					
Carbaryl[d]	Unexposed	246	Ref		0.55
	>0 – ≤380	89	1.30 (1.02, 1.67)	0.03	
	>380 – ≤2337	83	1.26 (0.97, 1.63)	0.09	
	>2337	73	1.19 (0.88, 1.62)	0.26	
Carbofuran	Unexposed	510	Ref		0.79
	>0 – ≤376	101	1.31 (1.06, 1.63)	0.01	
	>376 – ≤1370	74	0.98 (0.77, 1.26)	0.89	
	>1370	75	1.06 (0.83, 1.36)	0.63	
Organophosphate insecticide					
Chlorpyrifos	Unexposed	431	Ref		0.77
	>0 – ≤455	124	1.06 (0.86, 1.29)	0.59	
	>455 – ≤1848	111	0.95 (0.77, 1.17)	0.63	
	>1848	112	1.04 (0.85, 1.28)	0.70	
Coumaphos[e] ≤62 years	Unexposed	384	Ref		0.49
	>0 – ≤750	18	0.96 (0.60, 1.54)	0.86	
	>750	17	0.83 (0.51, 1.34)	0.44	
>62 years	Unexposed	281	Ref		0.02
	>0 – ≤750	25	1.41 (0.94, 2.13)	0.10	
	>750	24	1.64 (1.07, 2.48)	0.02	
Dichlorvos	Unexposed	638	Ref		0.20
	>0 – ≤539	45	1.47 (1.08, 2.00)	0.01	
	>539 – ≤3915	49	1.60 (1.19, 2.15)	0.00	
	>3915	36	1.25 (0.89, 1.75)	0.20	
Diazinon[d]	Unexposed	352	Ref		0.01
	>0 – ≤315	46	1.33 (0.98, 1.82)	0.07	
	>315 – ≤1218	49	1.43 (1.05, 1.93)	0.02	
	>1218	45	1.49 (1.07, 2.06)	0.02	
Fonofos	Unexposed	573	Ref		0.08
	>0 – ≤448	57	0.98 (0.74, 1.29)	0.89	
	>448 – ≤1680	77	1.30 (1.02, 1.65)	0.04	
	>1680	69	1.22 (0.95, 1.58)	0.13	
Malathion[d]	Unexposed	136	Ref		0.68
	>0 – ≤360	103	1.16 (0.89, 1.50)	0.27	
	>360 – ≤1395	143	1.48 (1.16, 1.88)	0.00	
	>1395	106	1.16 (0.89, 1.50)	0.27	
Parathion[d]	Unexposed	442	Ref		0.69
	≤868	28	1.48 (1.00, 2.18)	0.05	
	>868	18	1.08 (0.67, 1.75)	0.74	
Phorate[d]	Unexposed	323	Ref		0.74
	>0 – ≤315	66	1.18 (0.90, 1.56)	0.23	

[a]Split using tertile cut-offs or at the median of intensity-weighted lifetime days among exposed for each pesticide.
[b]Adjusted for sex, education, state, and smoking.
[c]*p*-Trend values were obtained using an ordinal variable coded with median values for each category.
[d]Belong to the 28 pesticides for which frequency and duration were asked only in the take-home questionnaire.
[e]HR allowed to vary by the median age (i.e., 62 years) for pesticides that did not meet proportional hazards assumptions ($p \leq 0.10$).
Note: Those who developed hyperthyroidism were censored at age of diagnosis. CI, Confidence Intervals; DDT, Dichlorodiphenyltrichloroethane; HR, Hazard Ratio.

Table 3. (*Continued.*)

Pesticide	Intensity-weighted days[a]	Cases	HR (95% CI)[b]	*p*-Value	*p*-Trend[c]
	>315 – ≤ 1176	50	0.91 (0.67, 1.23)	0.54	
	>1176	49	0.98 (0.72, 1.33)	0.91	
Terbufos	Unexposed	437	Ref		0.29
	>0 – ≤ 645	114	1.19 (0.96, 1.47)	0.11	
	>645 – ≤ 2400	109	1.10 (0.89, 1.36)	0.39	
	>2400	106	1.15 (0.93, 1.43)	0.20	
Permethrin (animals)	Unexposed	654	Ref		0.09
	>0 – ≤ 368	42	1.19 (0.87, 1.63)	0.28	
	>368 – ≤ 1418	41	1.19 (0.86, 1.63)	0.29	
	>1418	43	1.30 (0.95, 1.78)	0.10	
Permtherin (crops)	Unexposed	641	Ref		0.06
	>0 – ≤ 490	54	1.10 (0.83, 1.45)	0.52	
	>490	57	1.30 (0.98, 1.71)	0.06	
Carbon tetrachloride/carbon disulphide[d]	Unexposed	465	Ref		1.00
	>0 – ≤ 168	14	1.01 (0.59, 1.72)	0.98	
	>168	13	1.00 (0.57, 1.74)	0.99	
Ethylene Dibromide[d]	Unexposed	468	Ref		0.37
	>0 – ≤ 441	13	1.28 (0.73, 2.26)	0.39	
	>441	12	1.31 (0.72, 2.37)	0.38	
Methyl Bromide	Unexposed	713	Ref		0.44
	>0 – ≤ 320	42	1.09 (0.78, 1.52)	0.61	
	>320 – ≤ 1372	35	0.92 (0.64, 1.33)	0.66	
	>1372	32	0.88 (0.60, 1.28)	0.50	

associated with increased risk only for coumaphos among those aged >62 y and diazinon in the overall sample. Unlike organochlorines, organophosphate insecticides are nonpersistent chemicals with shorter half-lives. All four organophosphates [i.e., coumaphos, diazinon, dichlorvos (also a chlorinated compound), and malathion] were associated with higher prevalence of hypothyroidism in the previous investigation of the AHS male farmers (Goldner et al. 2013). No associations were seen for these insecticides in the female AHS spouses (Goldner et al. 2010). In the BEEA study, odds of subclinical hypothyroidism were elevated among those in the exposed quartiles of diazinon in comparison with unexposed, but without a dose–response trend (Lerro et al. 2017). In a study of Mexican floricultural workers, increasing levels of urinary dialkyl phosphates—measures of total organophosphate exposure—were associated with increasing levels of serum TSH and the TH total thyroxine (Lacasaña et al. 2010). Concurrent increases in both TSH and total thyroxine levels are somewhat contradictory to typical hormonal alterations observed in hypothyroidism (i.e., increase in TSH with normal TH levels for subclinical condition or decreased TH levels for overt disease); these findings were attributed to the potential of pesticides to act via multiple mechanisms.

Our findings of increased risk of hypothyroidism associated with the chlorophenoxy herbicides 2,4-D and dicamba are consistent with prior findings for prevalent hypothyroidism in male farmers; however, no association was previously observed for the herbicide glyphosate as it was in the present analysis (Goldner et al. 2013). These herbicides were not associated with increased prevalence of hypothyroidism in AHS spouses or subclinical hypothyroidism in the BEEA study (Goldner et al. 2010; Lerro et al. 2017). To our knowledge, no other human studies have examined these herbicides in relation to thyroid function. We did not detect elevated hypothyroidism risk for fungicides or fumigants, although fungicides belonging to the group ethylenebis (dithiocarbamate) (for example, maneb/mancozeb) have been suggested to elicit hypothyroid-like effects in animal (Axelstad et al. 2011; Mallem et al. 2006) and in human studies (Goldner et al. 2010; Steenland et al. 1997).

We also detected positive associations for permethrin use (on both animals and crops), with increasing risks with increasing exposure categories in our main analyses, although associations were not statistically significant. There is not much information from human studies (Jain 2016; Meeker et al. 2009), except for the finding that the pyrethroid metabolite *cis*-3-(2-2- dichlorovinyl) -2,2 - dimethylcyclopropane carboxylic acid (DCCA) was inversely associated with the thyroid hormone total triiodothyroinine in a cross-sectional study of 161 men from an infertility clinic (Meeker et al. 2009). Further, no association with permethrin use was seen in the BEEA study (Lerro et al. 2017).

Pesticides could alter thyroid function by several mechanisms, including interference with TH synthesis, transport, and hepatic and peripheral metabolism, and via immunomodulation/autoimmunity (Langer 2010; Miller et al. 2009), although there are limited toxicological studies illustrating these mechanistic pathways specifically in relation to pesticides. Organochlorine chemicals such as polychlorinated biphenyls and DDT and/or DDE have been shown to interfere with binding to as well as to decrease transthyretin, a TH transport protein, and enhance hepatic catabolism of THs (all likely resulting in reduction in serum TH levels) (Liu et al. 2011; Tebourbi et al. 2010; Van den Berg et al. 1991); these chemicals were also shown to bring about structural alterations in the thyroid gland (Collins and Capen 1980; Langer 2010; Tebourbi et al. 2010) and elicit autoimmune responses (Freire et al. 2013; Schell et al. 2009). In most animal studies, organochlorine insecticides (including DDT and lindane) are shown to decrease serum THs and increase TSH, suggesting hypothyroid-like effects (Brucker-Davis 1998). However, in birds, DDT has been shown to cause hypothyroidism in high exposure and hyperthyroidism in low exposure (Jefferies and French 1971), hinting that exposure-intensity may determine the nature of thyroid outcomes. As for organophosphates, varying findings [for example, decreased (Akhtar et al. 1996) as well as increased thyroxine for malathion (U.S. EPA 2015a), decreased thyroxine for diazinon (U.S. EPA 2015b)] have been reported in animal studies. Regarding mechanisms, diisopropylfluorophosphate (an acetylcholinesterase inhibitor like organophosphates and carbamates) has been shown to suppress TSH production potentially via pathways involving muscarinic and nicotinic receptors (Smallridge et al. 1991). Further, the organophosphate chlorpyrifos has been shown to produce thyroid alterations at doses not inhibiting acetylcholinesterase in mice (De Angelis et al. 2009), which

Table 4. Intensity-weighted lifetime days of use of fungicides and herbicides and hypothyroidism risk.

Pesticide	Intensity-weighted days[a]	Cases	HR (95% CI)[b]	p-Value	p-Trend[c]
Fungicides					
Benomyl	Unexposed	454	Ref		0.44
	>0 – ≤ 841	21	1.14 (0.73, 1.8)	0.56	
	>841	15	0.82 (0.48, 1.4)	0.46	
Captan[d] ≤62 years	Unexposed	375	Ref		0.85
	>0 – ≤ 9	15	0.85 (0.50, 1.42)	0.53	
	>9 – ≤ 192	12	0.79 (0.45, 1.41)	0.43	
	>192	16	1.02 (0.61, 1.69)	0.95	
>62 years	Unexposed	304	Ref		0.45
	>0 – ≤ 9	10	1.03 (0.55, 1.94)	0.93	
	>9 – ≤ 192	11	1.17 (0.64, 2.13)	0.61	
	>192	10	0.79 (0.42, 1.49)	0.46	
Chlorothalonil	Unexposed	768	Ref		0.18
	>0 – ≤ 588	21	1.10 (0.71, 1.71)	0.68	
	>588 – ≤ 3162	19	1.12 (0.70, 1.79)	0.63	
	>3162	11	0.64 (0.35, 1.18)	0.16	
Maneb/Mancozeb	Unexposed	452	Ref		0.98
	>0 – ≤ 457	6	0.48 (0.21, 1.09)	0.08	
	>457 – ≤ 2744	19	1.53 (0.94, 2.50)	0.09	
	>2744	11	0.93 (0.50, 1.72)	0.81	
Metalaxyl	Unexposed	415	Ref		0.57
	>0 – ≤ 312	22	0.69 (0.45, 1.07)	0.10	
	>312 – ≤ 1512	25	0.91 (0.59, 1.40)	0.66	
	>1512	26	1.11 (0.71, 1.72)	0.65	
Herbicide					
Alachlor	Unexposed	334	Ref		0.69
	>0 – ≤ 809	124	0.88 (0.71, 1.08)	0.22	
	>809 – ≤ 3132	186	1.33 (1.11, 1.60)	0.00	
	>3132	132	1.02 (0.83, 1.25)	0.87	
Butylate	Unexposed	346	Ref		0.49
	>0 – ≤ 455	51	1.10 (0.81, 1.48)	0.54	
	>455 – ≤ 1523	44	0.92 (0.67, 1.26)	0.59	
	>1523	51	1.14 (0.84, 1.53)	0.40	
Chlorimuron Ethyl[e]	Unexposed	340	Ref		0.52
	>0 – ≤ 245	55	1.09 (0.81, 1.45)	0.58	
	>245 – ≤ 744	46	0.92 (0.67, 1.25)	0.59	
	>744	49	1.12 (0.83, 1.52)	0.45	
Dicamba	Unexposed	325	Ref		0.10
	>0 – ≤ 572	146	1.24 (1.00, 1.54)	0.05	
	>572 – ≤ 2184	157	1.32 (1.07, 1.63)	0.01	
	>2184	144	1.29 (1.04, 1.59)	0.02	
EPTC[d] ≤62 years	Unexposed	324	Ref		0.57
	>0 – ≤ 315	40	1.38 (0.99, 1.93)	0.06	
	>315 – ≤ 1209	25	0.84 (0.55, 1.26)	0.40	
	>1209	33	1.16 (0.81, 1.67)	0.42	
>62 years	Unexposed	278	Ref		0.86
	>0 – ≤ 315	21	0.90 (0.58, 1.41)	0.66	
	>315 – ≤ 1209	28	1.25 (0.84, 1.85)	0.27	
	>1209	20	1.00 (0.63, 1.58)	0.99	
Glyphosate	Unexposed	158	Ref		0.95
	>0 – ≤ 686	221	1.27 (1.03, 1.56)	0.02	
	>686 – ≤ 2604	238	1.38 (1.12, 1.69)	0.00	
	>2604	197	1.17 (0.94, 1.45)	0.15	
Imazethapyr	Unexposed	434	Ref		0.88
	>0 – ≤ 341	115	0.97 (0.78, 1.21)	0.82	
	>341 – ≤ 1015	118	1.05 (0.84, 1.31)	0.65	
	>1015	103	1.01 (0.80, 1.27)	0.96	
Metolachlor[d] ≤62 years	Unexposed	219	Ref		0.79
	>0 – ≤ 720	59	0.87 (0.65, 1.17)	0.36	
	>720 – ≤ 2688	77	1.11 (0.85, 1.45)	0.44	
	>2688	68	1.02 (0.78, 1.35)	0.87	
>62 years	Unexposed	197	Ref		0.08
	>0 – ≤ 720	63	1.15 (0.86, 1.53)	0.36	
	>720 – ≤ 2688	55	0.99 (0.73, 1.35)	0.97	
	>2688	35	0.75 (0.52, 1.07)	0.11	

[a]Split using tertile cut-offs or at the median of intensity-weighted lifetime days among exposed for each pesticide.
[b]Adjusted for sex, education, state, and smoking.
[c]p-Trend values were obtained using an ordinal variable coded with median values for each category.
[d]HR allowed to vary by the median age (i.e., 62 years) for pesticides for which proportional hazards assumptions were not met ($p \leq 0.10$).
[e]Belong to the 28 pesticides for which frequency and duration were asked only in the take-home questionnaire.
Note: those who developed hyperthyroidism were censored at age of diagnosis. 2,4-D, 2,4-Dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-Trichlorophenoxyacetic acid; CI, Confidence Intervals; EPTC, S-Ethyl dipropylthiocarbamate; HR, Hazard Ratio.

Table 4. (*Continued.*)

Pesticide	Intensity-weighted days[a]	Cases	HR (95% CI)[b]	*p*-Value	*p*-Trend[c]
Paraquat[e]	Unexposed	428	Ref		0.39
	>0 – ≤ 588	37	0.92 (0.65, 1.30)	0.63	
	>588	30	0.84 (0.57, 1.25)	0.39	
Pendimethalin[e]	Unexposed	324	Ref		0.45
	>0 – ≤ 331	69	1.10 (0.84, 1.43)	0.49	
	>331 – ≤ 1274	40	0.67 (0.48, 0.94)	0.02	
	>1274	59	1.17 (0.89, 1.56)	0.27	
Petroleum[e]	Unexposed	380	Ref		0.77
	>0 – ≤ 504	38	1.03 (0.74, 1.45)	0.84	
	>504 – ≤ 2438	37	1.07 (0.76, 1.51)	0.69	
	>2438	36	1.05 (0.75, 1.48)	0.77	
Trifluralin	Unexposed	326	Ref		0.34
	>0 – ≤ 1008	133	1.02 (0.83, 1.25)	0.87	
	>1008 – ≤ 3828	166	1.25 (1.03, 1.53)	0.02	
	>3828	141	1.10 (0.90, 1.36)	0.34	
2,4-D	Unexposed	144	Ref		0.01
	>0 – ≤ 1274	188	1.21 (0.97, 1.52)	0.09	
	>1274 – ≤ 5104	223	1.30 (1.04, 1.63)	0.02	
	>5104	252	1.44 (1.16, 1.79)	0.00	
2,4,5-T[d,e] ≤62 years	Unexposed	213	Ref		0.16
	>0 – ≤ 480	18	0.84 (0.52, 1.36)	0.47	
	>480	12	0.68 (0.38, 1.23)	0.20	
>62 years	Unexposed	157	Ref		0.05
	>0 – ≤ 480	46	1.38 (0.99, 1.93)	0.06	
	>480	48	1.41 (1.02, 1.96)	0.04	
Atrazine[d] ≤62 years	Unexposed	132	Ref		0.30
	>0 – ≤ 1046	98	0.84 (0.64, 1.10)	0.20	
	>1046 – ≤ 4437	118	0.98 (0.76, 1.28)	0.90	
	>4437	97	0.86 (0.65, 1.12)	0.26	
>62 years	Unexposed	88	Ref		0.15
	>0 – ≤ 1046	77	1.03 (0.75, 1.41)	0.87	
	>1046 – ≤ 4437	104	1.25 (0.93, 1.69)	0.14	
	>4437	101	1.25 (0.93, 1.69)	0.14	
Cyanazine[d] ≤62 years	Unexposed	240	Ref		0.41
	>0 – ≤ 564	66	1.07 (0.81, 1.43)	0.62	
	>564 – ≤ 2268	51	0.82 (0.60, 1.13)	0.23	
	>2268	69	1.12 (0.85, 1.49)	0.42	
>62 years	Unexposed	174	Ref		0.80
	>0 – ≤ 560	54	1.14 (0.83, 1.58)	0.42	
	>560 – ≤ 2268	72	1.46 (1.09, 1.96)	0.01	
	>2268	45	1.08 (0.77, 1.53)	0.65	
Metribuzin[e]	Unexposed	297	Ref		0.65
	>0 – ≤ 315	66	0.96 (0.73, 1.27)	0.80	
	>315 – ≤ 1006	57	0.82 (0.61, 1.10)	0.18	
	>1006	70	1.07 (0.82, 1.40)	0.63	

may suggest the possibility that these chemicals may act via other distinct pathways and thus elicit divergent thyroid responses, depending on exposure intensity. Organophosphates including malathion and chlorophenoxy herbicides including 2,4-D have also been shown to interfere with transthyretin (Van den Berg et al. 1991).

Our study has several limitations. Thyroid disease information was based on self-reports and thus prone to misclassification. Also, because we asked participants about doctor-diagnosed thyroid disease rather than conducting cohort-wide screening, we undoubtedly missed some cases. In our validation effort, we found good agreement between hypothyroidism self-reports and medical records, with about 90% of hypothyroidism self-reports confirmed by medical records. The Nurses' Health Study II also found high agreement between self-reports and medical record reports, suggesting, hypothyroidism self-reports are reliable (Kang et al. 2013). The fact that we could replicate a known inverse association between smoking and hypothyroidism also supports reliability of self-reports (Wiersinga 2013). Concordant results from sensitivity analyses restricted to those consistently reporting disease or reporting disease-specific treatments also provide reassurance. In this prospective cohort study, we expect any disease misclassification to be nondifferential (Blair et al. 2011). Still, as we asked about doctor-diagnosed thyroid disease, and individuals with greater access to or use of healthcare may have had higher odds of having existing thyroid disease diagnosed, if pesticide use is related to healthcare access, systematic bias is possible.

Further, we were not able to assess associations with more specific conditions (namely, overt, subclinical, or autoimmune hypothyroidism), which could have shed more light on disease-risk severity and mechanisms. As participants are less likely to have in-depth knowledge about their disease or diagnostic tests, it would have required cohort-wide comprehensive assessment of thyroid function biomarkers to differentiate subclinical versus overt thyroid disease or to distinguish autoimmune disease from other diagnoses.

Pesticide use information was also based on self-reports. Although prior investigations show that AHS farmers provided fairly reliable and plausible pesticide use information (Blair et al. 2002; Hoppin et al. 2002), some exposure misclassification is inevitable. Whereas any exposure misclassification is likely to be nondifferential and would tend to bias effect estimates towards null for binary exposures, for intensity-weighted analyses with

more than two categories, it is possible that effect estimates would have been biased toward each other, attenuating estimates for higher exposure categories and inflating estimates for lower exposure categories (Rothman et al., 2008). Indeed, for intensity-weighted analyses for some pesticides, we observed that HRs were of similar magnitudes across all the exposure categories without an evident increasing dose–response. Still, we cannot exclude the possibility of nonmonotonic dose–response or the idea that, for autoimmune thyroid disease, the response may be idiosyncratic with no clear dose–response.

Further, in the current analysis, we considered pesticide use up to enrollment, but did not account for pesticide use that occurred during follow-up. This approach may not be a concern for organochlorines and other phased-out pesticides; however, for those pesticides with ongoing use, it likely resulted in some degree of exposure misclassification. Furthermore, the study participants were exposed to multiple pesticides, and individuals exposed to certain mixtures of pesticides may have greater disease risk. Future research is warranted on risk of incident hypothyroidism in relation to proximal (Phases 2 and 3) exposures and considering multiple pesticide exposures. Last, given the exploratory nature of the study, we did not adjust for multiple comparisons, and therefore some of these associations may be chance findings.

Our study also has several strengths, including its large sample size, prospective design, and comprehensive pesticide use information. Specifically, we used intensity-weighted lifetime days of pesticide use that incorporated determinants of exposure, such as personal protective use and work/hygiene practices, which correlate better with urinary biomarkers in comparison with other, cruder measures (Blair et al. 2011). Lastly, analyses using inverse probability weights produced results similar to the main analyses, suggesting that selection bias due to loss to follow-up is less likely.

In summary, our findings on pesticide-related hypothyroidism risk provide further evidence for specific pesticides' thyroid-disruptive properties. As many of these pesticides are still being used in agriculture, and the current study is one of the few to report associations with incident thyroid diseases, confirmation of these findings in future longitudinal studies is very important. Further, few mechanistic studies elucidate pesticides' thyroid-disruptive properties; therefore, further toxicological studies are warranted.

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